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Purpose of review
The clinical utility of HDLs has been scrutinized upon the publication of Mendelian randomization studies showing no effect of HDL-cholesterol (HDL-C) modifying variants on cardiovascular disease (CVD) outcome. The failures of randomized controlled HDL-C-directed intervention trials have further fueled this skepticism. This general criticism originates from oversimplification that has equated 'HDL-C' with 'HDL' and misconceived both as the 'good cholesterol'.

Recent findings
HDL particles are heterogeneous and carry hundreds of different lipids, proteins, and microRNAs. Many of them but not cholesterol, that is, HDL-C, contributes to the multiple protective functions of HDLs that probably evolved to manage potentially life-threatening crises. Inflammatory processes modify the composition of HDL particles as well as their individual protein and lipid components, and, as a consequence, also their functionality. Gain of dominant-negative functions makes dysfunctional HDL a part rather than a solution of the endangering situation. Quantification of HDL particle numbers, distinct proteins or lipids, and modifications thereof as well as bioassays of HDL functionality are currently explored toward their diagnostic performance in risk prediction and monitoring of treatment response.

Summary
Any successful clinical exploitation of HDLs will depend on the identification of the most relevant (dys)functions and their structural correlates. Stringent or prioritized structure-(dys)function relationships may provide biomarkers for better risk assessment and monitoring of treatment response. The most relevant agonists carried by either functional or dysfunctional HDLs as well as their cellular responders are interesting targets for drug development.

Keywords
cholesterol efflux, dysfunctional HDL, innate host defense, reverse cholesterol transport, sphingosine-1-phosphate bioassay

INTRODUCTION

Crises are 'specific, unexpected, and nonroutine events or series of events that [create] high levels of uncertainty and threat or perceived threat to an organization's high priority goals.' [1]. This definition is fulfilled by the role and fate of HDLs in both the life of an organism and medical research and development: HDLs help to prevent or manage crises of the organism caused, for example, by overload with cholesterol or xenobiotics, infectious or sterile inflammation, or injury [2]. For several decades, these properties as well as the association of low HDL-cholesterol (HDL-C) with increased cardiovascular risk [3,4] have generated considerable scientific interest in structure, function, metabolism, and regulation of HDL to exploit HDL for treatment and prevention of cardiovascular diseases (CVDs). Recent results of two lines of clinical or epidemiological research, however, led to a general critique of HDL's causal role in the pathogenesis of atherosclerotic CVD and hence suitability as a therapeutic target. First, in randomized controlled trials, HDL-C increasing drugs – namely fenofibrate, nicotinic acid, and three inhibitors of cholesteryl ester transfer protein (CETP) – did not reduce cardiovascular event rates beyond statins [5]. Second, in Mendelian randomization studies, several polymorphisms or rare mutants were found associated with differences in HDL-C but not in cardiovascular risk [6]. These criticisms overlook the nonlinear relationship between HDL-C and cardiovascular risk

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KEY POINTS

- HDLs exert multiple functions that contribute to host defense from biological or chemical harm. These functions are exerted by HDL-holoparticles as well as specific agonists carried by HDL, for example, S1P. These properties make HDL interesting therapeutic targets even beyond prevention of CVDs.
- Inflammatory conditions modify both the composition and the structural components of HDLs. The resulting dysfunctional HDL particles not only lose protective functions but also gain noxious properties.
- Neither physiological function nor pathological dysfunction is recorded by the measurement of HDL-C. Novel biomarkers are currently searched and validated in clinical or epidemiological studies toward their potential to aid in drug development and improve risk prediction and monitoring of treatment response. Candidate biomarkers include HDL particle numbers, serum amyloid A, apoC-III, S1P, certain post-translational modifications of apoA-I, and cholesterol efflux capacity.
- The systematic elucidation of structure-function relationships of functional and dysfunctional HDL, for example, by a systems biology approach, may help to discover novel biomarker candidates as well as rationales and targets for therapeutic exploitation of HDL.

which according to meta-analyses is significant below the seventh decile only [3], the unsuitable design of most intervention studies which did not define HDL-C levels as inclusion/exclusion criteria, and the pleiotropic effects of the investigated drugs, which by also modulating plasma levels of LDL-cholesterol (LDL-C) and/or triglycerides do not allow any conclusion on the contribution of HDL-C to trial failure [7]. Most importantly, however, the cholesterol in HDL which is measured by HDL-C is neither the mediator nor the reporter of HDL function [2].

CRISIS PREVENTING AND SOLVING PROPERTIES OF HDLs

HDLs are heterogeneous and multimolecular complexes of hundreds of different molecules (proteins, lipids, microRNAs, and additional amphiphilic or lipophilic small molecules) [2]. Plasma concentrations of these molecules span four to five orders of magnitude and range from submicromolar [lipid transfer proteins, apoL1, sphingosine-1-phosphate (S1P)] to millimolar (cholesterol, phosphatidylcholine) [2]. Assuming HDL particle concentration of 20 μmol/l, an average HDL particle carries 50–100 molecules of cholesterol or phosphatidylcholine but less than 5% of the particles carry one molecule of the minor constituents. Already these numbers make clear that HDL-C cannot mirror the diversity and functionality of HDLs.

HDLs protect the function and survival of the organism by four different principle mechanisms.

Cholesterol efflux and reverse cholesterol transport

As the classical function, HDLs mediate transport of cholesterol from macrophage-foam cells to the liver for biliary excretion. HDLs elicit cholesterol efflux from cells via interaction with either ATP-binding cassette (ABC) transporters or scavenger receptor B1 (SR-B1) [2,8]. ABCA1-mediated phospholipid and cholesterol efflux was originally proposed to be elicited by prebeta1-HDL, that is, lipid-free apoA-I. The thereby generated discoidal nascent HDL particles as well as spherical mature HDLs produced by the subsequent esterification of cholesterol through lecithin:cholesterol acyltransferase (LCAT), were postulated to interact with SR-B1 and ABCG1 [2,8]. Recent systematic studies revealed that small dense HDL particles and lipid-free apoA-I are the most efficient mediators of cholesterol efflux [9▪], whereas ABCG1 did not contribute much to cholesterol efflux to small HDLs and little to cholesterol efflux to large HDLs. It rather appeared to mediate transport of cholesterol from intracellular pools to the plasma membrane [9▪].

Reverse cholesterol transport (RCT) is of special relevance for the removal of cholesterol from macrophages [2,8,10▪]. These cells of the innate immune system can bypass the down-regulated LDL receptor and take up modified lipoproteins through scavenger receptors, cells, and their debris as well as cholesterol crystals by different mechanisms of engulfment (phagocytosis, efferocytosis, pinocytosis) [10▪]. Cholesterol-loaded macrophage foam cells exert several proinflammatory activities. Thus, cholesterol loading of macrophages may have evolved to support host defense to combat acute infections and injuries [10▪]. However, if not resolved, this reaction propagates the pathogenesis of chronic inflammatory diseases, including atherosclerosis [10▪]. Cholesterol efflux by HDLs was shown to interfere with cytokine release. HDL-mediated cholesterol efflux may hence have evolved to resolve inflammation. In this regard, the opposite handling of cholesterol by LDL and HDL in inflammation is in analogy with the opposite action of different products of lipoxygenases and cyclooxygenases in inflammation, that is, lipoxins vs. leukotrienes and resolvins vs. prostaglandins, respectively [11].

Cholesterol efflux and RCT are probably also important means to compensate the dynamic and dramatic changes of cholesterol content in adipocytes elicited by the shrinkage of biomembranes surrounding lipid droplets during lipolysis [12]. In cells with less extreme alterations of cholesterol content, subtle HDL-induced changes in cellular cholesterol homeostasis have been associated with several changes of cellular differentiation and functions [10▪,13].

Signal transduction

HDLs elicit signal transduction processes which regulate the differentiation and egress, proliferation, survival, and function of various cell types [14]. In the context of host defense, the convergence of different HDL-induced cellular responses help to limit the inflammatory action of myelocytes and lymphocytes [11,15▪▪], provide integrity and functionality of endothelial barriers [16], secure energy homeostasis by stimulating insulin synthesis and secretion by pancreatic beta cells as well as glucose uptake by adipocytes and myocytes [17,18], stimulate angiogenesis [19], limit organ damage, and facilitate wound healing [20].

The cellular responses happen as the result of altered cholesterol homeostasis in specific plasma membrane domains, or specific agonist-receptor interactions activating signal transduction cascades. In this regard, HDL/SR-BI and apoA-I/ABCA1-interactions appear ambiguous. For example, the interaction of HDLs with SR-B1 activates kinases via its PDZ domain [14] or elicits cellular responses by altering the cholesterol content of specific plasma membrane domains that are enriched with signaling molecules such as endothelial nitric oxide synthase (eNOS) in caveolae of endothelial cells [13]. Likewise, the interaction of apoA-I with ABCA1 has been proposed to elicit cellular responses such as facilitated insulin secretion from beta cells or inhibited cytokine secretion from macrophages both indirectly by altering cellular cholesterol homeostasis via cholesterol efflux and directly by eliciting signal transduction, for example, through recruitment of heterotrimeric G-protein $G\alpha_S$ subunits or induction of signal transducer and activator of transcription 3 phosphorylation [14,17,18].

S1P is an example of less ambiguous agonist/receptor interactions: S1P is enriched in about 5% of plasma HDL particles by binding to apoM and interacts with five different G-protein coupled S1P receptors [14,21]. Apart from mediating the quantitative enrichment of S1P in HDLs, apoM does also act as a chaperone that modulates the quality of S1P/S1P receptor interactions. ApoM-bound and hence HDL-carried S1P but not albumin-bound S1P was found to inhibit the differentiation, proliferation, and egress of murine lymphocytes as well as the suppression of NF-κB and intercellular adhesion Molecule 1 (ICAM-1) in endothelial cells [10▪,17,22▪]. In endothelial cells S1P promotes nitric oxide production, junction closure, cell proliferation and migration, as well as angiogenesis and inhibits apoptosis as well as TNFα-induced expression of vascular cell adhesion molecules [12,21]. S1P also promotes the survival of cardiomyocytes, hearts, and kidneys which are exposed to hypoxia, ischemia-reperfusion injury, or toxic drugs [14,21,23]. Some of these activities are also exerted by S1P-free reconstituted HDLs. However, the addition of S1P enhances the ability of S1P-free reconstituted HDL to protect hearts from ischemia reperfusion injury [23]. Moreover, HDLs were found to stimulate S1P efflux from erythrocytes in both apoM-dependent and apoM-independent manner. Initially, S1P-free or S1P-poor HDLs may hence be loaded with S1P during incubation with cells [24,25▪]. As the alternative to this mediation of autocrine and paracrine effects, S1P-free and S1P-containing HDLs may elicit identical cellular responses by alternative mechanisms. Such mechanistic redundancy, however, is another indication for an important physiological role of HDLs.

Inactivation of extracellular biohazards

By its amphiphilic nature, HDLs can bind bacterial lipopolysaccharides, oxidized lipids as well as xenobiotics [26]. In this respect, it is interesting to note that HDLs have been isolated from several body fluids that separate cells from their external environment, for example, tears, nasal fluid, saliva, and bronchial fluid. [26] The intestine is even producing HDLs by itself [27]. Potentially hazardous molecules, including cholesterol are either eliminated by reverse transport to the liver or inactivated directly on the surface of HDLs [26]. The best investigated example for the latter situation is the hydrolysis of oxidized phospholipids, which are transferred by CETP or phospholipid transfer protein from LDLs to HDLs [28], by paraoxonase 1 (PON1), lipoprotein associated phospholipase A2, and lecithin:cholesterol acyltransferase. Notably, the antiatherogenic effect of PON1 is supported by data from clinical and epidemiological observational studies as well as genetic mouse models with knock-out or overexpression of PON1 [29].

Apart from inactivating or eliminating potentially noxious agents, the amphiphilic structure and specific molecules carried by HDLs also help to counteract downstream adverse effects of

inflammation, for example, complement activation, coagulation, and platelet aggregation. Some of these effects are exerted by apoA-I/phosphatidylcholine complexes, for example, the prevention of von Willebrand factor's self-association [30] or complement activation by cholesterol crystals [31]. Others are mediated by specific inactivators or inhibitors carried by HDLs, for example, the complement lysis inhibiting factor clusterin, antiproteolytic serpins, or the anticoagulant protein S [32,33].

Cellular uptake of HDLs or HDL components

HDLs deliver cargo to cells either by selective uptake, that is, independently of the entire particle, or via holoparticle uptake. Apart from mediating selective uptake of lipids from HDLs into liver and steroidogenic organs, SR-BI also facilitates the transfer of other HDL-associated molecules to cells, for example, microRNAs (miRNAs). In-vitro HDLs were found to deliver miRNAs to hamster baby kidney cells and human hepatocytes through SR-BI [34]. HDL-mediated delivery of miR-223 into endothelial cells was found to suppress ICAM-1 [35]. However, these findings are in contrast to those by Wagner *et al.* [36] who confirmed the presence of low copy numbers of miRNAs in HDLs, but found no evidence that HDLs deliver functionally relevant amounts of miRNAs into endothelial cells, smooth muscle cells, or monocytes [36]. Wagner *et al.* [36] found 10 000 copies of miR223 per μg HDL, that is, about 10 pmol HDL particles. The concentration of miR223 is hence 7 to 8 orders of magnitude below HDL particle concentration. The delivery of sufficient numbers of miRNA molecules for interference with several RNA molecules will require the interactions of a large number of HDL particles with a single cell. If at all this will more likely happen by autocrine or paracrine regulation of neighboring cells, that is locally, by using HDLs as a shuttle of secreted miRNAs as it has been described for exosomes [37], rather than by endocrine regulation of distant organs.

HDLs are also internalized by various cells, including hepatocytes, monocyte-derived macrophages, enterocytes, and endothelial cells [38]. ApoA-I has been shown to stimulate the uptake of HDL particles into hepatocytes and endothelial cells by activating the ectopic F1-ATPase/P2Y receptor axis [39]. In mice, the knock-out of P2Y13, which activates hepatic HDL uptake, impaired RCT from macrophage to feces and enhanced atherosclerosis despite counter-balanced SR-BI upregulation [40▪]. The potential clinical relevance of this F1-ATPase/P2Ys axis in humans is indicated by the identification of serum F1-ATPase inhibitor (IF1) as an independent determinant of HDL-C and coronary heart disease (CHD) risk [41].

In mice, the cubilin/megalin complex mediates the uptake of HDL-like particles from the primary urine into tubular epithelial cells of the kidney. The knock-out of cubilin leads to decreased plasma levels of HDL-C in mice suggesting that this pathway may rescue some HDLs from renal degradation [42]. Interestingly, the knock-out of megalin or proteins regulating tubular endocytosis as well as the knock-out of apoM, which is a ligand of cubilin/megalin, led to the urinary excretion of S1P [24].

Our and other labs provided evidence that HDL-holoparticle uptake plays an important role for transport of HDLs through endothelial barriers, for example, into the vascular wall or into the brain [43,44]. Cultured endothelial and epithelial cells were observed to store HDLs or HDL components within intracellular vesicles [43,45▪] suggesting that HDLs or its components also fulfil intracellular functions. In fact, internalization of HDL is a prerequisite for the killing of *Trypanosoma brucei* by apoL1 [46]. Recently, internalization of HDLs with concomitant recruitment of phosphorylated IκB kinase into autophagosomes was suggested as a means of preventing the TNFα-mediated activation of NF-κB in the enterocyte cell line T84 and as a mechanism by which knock-out of apoA-I and transgenic overexpression of human apoA-I enhanced and suppressed, respectively, the intestinal inflammation in a mouse model of inflammatory bowel disease [45▪].

PERSISTING CRISIS LEADS TO HDL DYSFUNCTION

Very similar to the classical components of the immune system, the functionality of HDLs appears to be compromised by acute and chronic inflammatory conditions as well as metabolic decompensation, including infections, rheumatic and autoimmune diseases, CHD, diabetes, chronic kidney disease, or familial hypercholesterolemia [2,47]. The broad spectrum of HDL dysfunctions include reduced capacities to stimulate cholesterol efflux from macrophages, to inhibit LDL oxidation, apoptosis and, in endothelial cells, nitric oxide production, monocyte chemoattractant protein 1 as well as VCAM expression [2,16,47]. The structure-function relationships of HDL dysfunction (but also physiological function) are not comprehensively elaborated. Several dysfunctions have been associated with different molecules within HDLs. For example, endothelial HDL dysfunctions

have been assigned to the loss of S1P, plasmalogens, or clusterin, enrichment with serum amyloid A (SAA), apoC-III, or symmetric dimethylarginine, as well as oxidative modifications of apoA-I or phospholipids [2,16,25▪,47–50,51▪]. Differences in cholesterol efflux capacity (CEC) have been attributed to alterations in HDL particle number, HDL subclass distribution, the kinetics of HDL/apoA-I exchange, in phospholipid composition, the accumulation of SAA, or post-translational modifications of apoA-I [2,47,52–59,60▪]. As yet, villains and bystanders are not unequivocally discriminated.

In bioassays, most dysfunctions are recorded as reduction of normal functionality [2,47]. Beyond this apparent loss of function, HDLs of patients with CHD or chronic kidney disease were found to inhibit rather than stimulate nitric oxide production because they gained the ability to interact with the lectin-like oxidized LDL receptor LOX-1 and the Toll-like receptors TLR2 and TLR4, respectively, and thereby induced the phosphorylation of inhibitory rather than activating sites in eNOS [49,50]. Also SAA enrichment was found to generate noxious HDLs which in endothelial cells inhibit nitric oxide production and stimulate the production of reactive oxygen species as well as the expression of vascular cell adhesion molecule 1 [51▪]. These findings raise the question whether also apparent loss-of-functions are caused by dominant negative effects. For example, the myeloperoxidase-mediated oxidation of Tyr-192 or Trp-72 residues in apoA-I which has been related to decreased CEC, affects about 20% of apoA-I in atherosclerotic plaques but only 0.02% of apoA-I molecles in plasma of CHD patients [56,57]. By loss of function, these modifications may limit macrophage cholesterol efflux locally within the arterial wall and contribute to the pathogenesis of atherosclerosis. However, the quantitatively very minor alterations can only markedly reduce CEC of total or apoB-free plasma, if they inhibit the cellular cholesterol efflux machinery. Interestingly, the injection of myeloperoxidase-oxidized apoA-I into mice decreased HDL-C levels and RCT from macrophages into plasma, whereas injection of nonoxidized apoA-I increased both HDL-C and RCT [58]. Likewise, the injection of HDLs of CKD patients increased blood pressure in mice [50]. The decreased CEC of HDLs enriched with SAA was explained by enhanced affinity to proteoglycans which prevents the access of HDLs to the plasma membrane [60▪]. These examples are the first indications that gain of dysfunction converts originally protective HDLs into noxious particles.

CRITICAL FINDINGS IN EPIDEMIOLOGICAL AND CLINICAL RESEARCH

By contrast to previous studies [3,4], several recent studies did not find the inverse association between HDL-C and CHD events in patients with chronic or acute CHD [60▪,61,62]. Also a systematic comparison of associations between lipid risk factors and CHD events in three US-American population studies found the association of HDL-C (but also other lipid risk factors) with incident CHD weakened in the contemporary studies with baseline levels assessed between 2003 and 2007 as compared with the older atherosclerosis risk in communities (ARIC) study (with baseline levels assessed between 1988 and 1997) [63▪]. The authors made the more prevalent statin use responsible for this secular trend. An alternative or additional explanation may be the changes in the methodology of HDL-C determination: the nowadays used homogenous assays for the determination of HDL-C (but also LDL-cholesterol) are notoriously inaccurate especially in dyslipidemic samples [64,65].

The negative results of randomized outcome trials on the add-on statin therapies with fenofibrate, niacin, or CETP inhibitors are frequently used arguments against the causal role and hence therapeutic suitability of HDLs [6]. However, in the randomized trials the effects of niacin or fenofibrate on HDL-C levels were rather modest (<10%). Moreover, with the exception of dalcetrapib [59], all drugs also beneficially affect LDL-C and lipoprotein(a) (niacin, torcetrapib, evacetrapib) and triglycerides (niacin and fenofibrate) [7]. These intervention trials hence do not allow any conclusion on the causal contribution of HDL-C to their failure. According to a recent meta-analyses, fibrates and niacin prevented cardiovascular events in the absence of statins but not if combined with statins [6]. Post hoc analyses revealed that patients with low HDL-C (<35 mg/dl/ < 0.9 mmol/l) and hypertriglyceridemia (>200 mg/dl/ > 2.3 mmol/l) benefit from treatment with fibrates independently of whether or not they were combined with statins [66]. Genetic post hoc analyses of the dal-OUTCOME study identified genetic differences in the adenylate cyclase 9 locus (ADCY9) as discriminators of dalcetrapib's efficacy [67▪]. Dalcetrapib treatment decreased the cardiovascular event rate by 39% in patients with the AA genotype at rs1967309 of the ADCY9 locus but increased cardiovascular event rates by 27% in patients with the GG genotype [67▪]. Randomized trials in such preselected patient groups are needed to prove that personalized treatment of patients with the low HDL-C/hypertriglyceridemia syndrome with fibrates or with the AA genotype at rs1967309 with a CETP inhibitor is effective.

Mendelian randomization studies provided evidence that associations with CHD risk of LDL-C, lipoprotein(a), and triglycerides but not with HDL-C are causal. Genetic mutants or polymorphisms that are associated with differences in HDL-C were not found associated with any differences in the prevalence or incidence of CHD events [5]. CETP polymorphisms are an exception in this regard. Carriers of CETP alleles associated with low CETP activity and, as the consequence, higher levels of HDL-C as well as lower levels of LDL-C and triglycerides have reduced risk of CVD [68,69]. It has been argued that these associations are driven by the effects of the polymorphisms on triglycerides and LDL-C [68]. However, it is important to recall that these polymorphisms like pharmacological CETP inhibitors primarily affect CETP activity and only secondarily lipid traits. The failures of torcetrapib, dalcetrapib [6,62], and possibly also evacetrapib [70] to reduce CHD event rates indicate that inborn and acquired decreases in CETP activity may have a different impact on CHD. This example points to limitations of the genetic strategy to predict outcome of pharmacological intervention.

Observational studies also found inverse associations between HDL-C and risks of ischemic stroke [3,71], progression of aortic aneurysm [72], incidence of diabetes mellitus [17,73,74], breast cancer [75] and colorectal cancer [76], as well as fatality of sepsis [77]. Although statistically independent of potential confounders, these associations also raise questions on causality. Two Mendelian randomization studies came to opposite conclusions on the causal association of low HDL-C with increased risk of diabetes mellitus: In the Copenhagen City Heart Study HDL-C levels but not genetic polymorphisms that influence HDL-C were found associated with incident diabetes mellitus [73]. By contrast, a meta-analysis of 140 single nucleotide polymorphisms known to affect plasma lipids found both measured and genetically determined elevations of HDL-C levels were found associated with decreased risk of diabetes mellitus [74]. Age-related macular degeneration provides an opposite example, where risk increases with increasing HDL-C [78]. The 442Gly encoding allele of the CETP gene which increases HDL-C and decreases CHD risk was found associated with elevated risk of age-related macular degeneration in an East Asian population [79].

STRATEGIES TO COPE WITH THE CRITICAL CLINICAL UTILITY OF HDLs

As HDL-C is no longer accepted as a surrogate marker for drug development, biomarkers that reflect HDL function are searched. Such biomarkers must not only show diagnostic or prognostic superiority over HDL-C as well as modifiable risk association but they must also be technically feasible in terms of preanalytical requirements, accessibility, and turnaround time as well as analytically well controlled in terms of precision, accuracy, and traceability [80,81]. As yet most criteria are either not fulfilled or not proven by novel HDL biomarkers.

HDL particles and subclasses

Particle numbers and size subclasses of HDLs can be determined by NMR spectroscopy and ion mobility. Nondenaturing gradient gel electrophoresis and agarose gel electrophoresis differentiate HDL subclasses by size and charge, respectively. The subclasses as determined by the different technologies cannot be simply interconverted but a consensus was reached that distinguishes five HDL subclasses by size [82]. By contrast to HDL subclasses, HDL particle numbers (HDL-P) as determined by NMR showed rather consistent inverse associations with risk of CHD events [83]. However, only some studies showed superiority of HDL-P over HDL-C (multiple risk factor intervention trial, multi-ethnic study of atherosclerosis (MESA), Dallas Heart Study, the Rosuvastatin arm of JUPITER) [84–87], whereas others did not (Women's Health study, community-based cohort study (CMCS) Beijing project, EPIC Norfolk, and the placebo arm of JUPITER) [87–90]. Interestingly, the CMCS Beijing project found the HDL-C/HDL-P ratio positively associated with the progression of carotid atherosclerosis [89]. This observation was also made in MESA and may indicate dysfunction of cholesterol-overloaded HDL particles or impairment in RCT. This is also indicated by the association of cholesterol-enriched particles with increased risk of CHD and reduced CEC in patients with high HDL-C levels [55]. Although MESA proved superiority of HDL-P, the use of HDL-P together with LDL-P did not improve overall risk prediction by the American Heart Association/American College of Cardiology calculator score [91]. Also of note, in the JUPITER study, HDL-P as determined by NMR but not as determined by ion mobility was associated with incident CHD [92]. Finally, it is important to note that most published data from outcome studies were obtained by the NMR method developed by LipoScience which is now commercialized as LipoProfile. At least three alternative NMR-based methods for HDL-P determinations use different algorithms for the analysis of NMR signals [93–95]. Two of them are also commercialized (LipoScale and LipoFit) [93,94]. LipoProfile and LipoScale yield HDL-P concentrations that show mean deviations of more than

60% from each other as well as discrepant correlations with HDL-apoA-I levels [93]. Systematic method comparisons and large observational studies are needed for all methods to harmonize HDL-P determination and to prove the diagnostic value of HDL-P independently of the method used.

Bioassays of HDL function

CEC, HDL-induced nitric oxide and superoxide production in endothelial cells as well as serum PON-1 activity show good precision within specialized labs [95,96,97▪,98] but accuracy and agreement of measurements between labs are unknown because of lacking reference material and absence of systematic external quality assessment surveys. Efflux of radiolabeled cholesterol and efflux of a fluorescent cholesterol analogue from J774 cells showed rather poor agreement with a coefficient of correlation of 0.52 [97▪]. Nevertheless, upon either method low CEC from J774 cells predicted cardiovascular events in three prospective cohort studies [97▪,99▪,100▪]. By contrast, CEC as determined by the use of RAW cells or THP1 cells as the cholesterol donors did not show the inverse association with cardiovascular events during the follow-up of patients who underwent diagnostic coronary angiography and kidney transplantation, respectively [101,102▪]. Rather by contrast, CEC determined in the presence of RAW cells was positively associated with the incidence of cardiovascular events, although it showed very good agreement with CEC determined from J774 cells ($r = 0.92$) [101]. In the kidney-transplanted patients, CEC from THP1 cells was independently associated with graft survival but not with cardiovascular events or mortality [102▪]. The increase in CEC but lack of clinical efficacy upon treatment with dalcetrapib or evacetrapib [103,104] indirectly question the utility of CEC to aid in drug development or monitoring of treatment success. Laborious and artifact-prone ultracentrifugation [105] limits the feasibility of bioassays such as recording of endothelial and anti-inflammatory functionalities in large sample numbers of clinical or epidemiological studies. Nevertheless, systematic head to head comparisons are needed to establish the HDL functionality which has the strongest association with CHD and treatment response and is, therefore, likely most relevant for the antiatherogenicity of HDLs.

HDL components

Apart from HDL-C, apoA-I has been most extensively investigated for its association with incident CHD. Overall apoA-I and HDL-C appear to have similar prognostic values [3,4]. Meta-analyses of statin trials revealed that on-treatment changes of apoA-I levels but not on-treatment changes of HDL-C are associated with outcome. However, the association is too weak for clinical utility [4].

Structure-function relationships of HDLs' function and dysfunction are not comprehensively elaborated. Most of them have been detected by explorative parallel functional, proteomic, lipidomic, or transcriptomic characterization of HDLs in relatively small case-control studies [25▪,48–50,51▪,56,57]. Only a few of them have been validated in larger longitudinal studies. The significant inverse association between PON1 activity and CHD was shown in several studies. It appears to be stronger in diabetic patients than in nondiabetic subjects [29]. In the Physician's Health and Nurses Health Studies, cholesterol in apoC-III-containing HDLs showed a positive association with incident CHD, whereas cholesterol in apoC-III-free HDLs showed the expected inverse association [106]. In diabetic patients on hemodialysis, a high SAA content of HDLs was associated with increased risk of cardiovascular events and death [107]. Interestingly, in the same study, cohort levels of apoA-II but not apoA-I or HDL-C were predictors of cardiovascular morbidity and total mortality [108]. In the LURIC study of 3310 patients undergoing coronary angiography, plasma levels of SAA and HDL-C were found to interact: in patients with plasma SAA levels below the 80th percentile, HDL-C showed the expected inverse association with all-cause and cardiovascular mortality. In patients with elevated SAA levels, the opposite associations were found [51▪]. Based on these findings, the authors derived a formula to calculate 'biologically effective HDL-C'. In two replication studies, one on the hemodialysis patients mentioned before and the other in the population-based cooperative health research in the region of Augsburg cohort, 'biologically effective HDL-C' but not measured HDL-C predicted outcome [51▪].

CONCLUSION

By their many functions in innate host defense, HDLs remain interesting targets for the clinical management of diseases, even beyond CVD. However, any successful clinical exploitation will depend on the identification of the most relevant (dys)functions and their structural correlates. This needs a study that comprehensively characterizes HDLs of healthy and diseased donors for structure (particle size by NMR and particle composition by proteomics, lipidomics, miRNA transcriptomics) and function (diverse bioassays, e.g., on CEC from macrophages and eNOS activation in endothelial

cells). Combined with bioinformatics such a systems biological approach may prioritize (dys)functions and components which are most strongly correlated with each other and most closely associated with disease states. The most stringent structure-(dys)function-phenotype relationships will provide biomarker candidates to improve risk assessment and monitoring of treatment response. The most relevant agonists of either functional or dysfunctional HDLs are interesting targets for drug development, either directly themselves or indirectly by unravelling their downstream cellular responders as therapeutic targets. Mediators of physiological protective functions would need activation, whereas mediators of HDL dysfunction would need inhibition. Lost functional HDL components would need replenishment, for example, by HDL mimetics [109] or activation. Mediators of dominant negative dysfunctions must be either eliminated or inhibited in formation or action. Any therapy that prolongs the residence time of HDLs with gained dysfunction will be rather harmful, even if it increases HDL-C levels.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Seeger MW, Sellnow TL, Ulmer RR. Communication, organization, and crisis. Communication Yearbook 1998; 21:231–275.
2. Annema W, von Eckardstein A. High-density lipoproteins. Multifunctional but vulnerable protections from atherosclerosis. Circ J 2013; 77:2432–2448.
3. Emerging Risk Factors Collaboration. Di Angelantonio E, Sarwar N, *et al.* Major lipids, apolipoproteins, and risk of vascular disease. JAMA 2009; 302:1993–2000.
4. Boekholdt SM, Arsenault BJ, Hovingh GK, *et al.* Levels and changes of HDL cholesterol and apolipoprotein A-I in relation to risk of cardiovascular events among statin-treated patients: a meta-analysis. Circulation 2013; 128:1504–1512.
5. Keene D, Price C, Shun-Shin MJ, Francis DP. Effect on cardiovascular risk of high density lipoprotein targeted drug treatments niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117 411 patients. BMJ 2014; 349:g4379.
6. Jansen H, Samani NJ, Schunkert H. Mendelian randomization studies in coronary artery disease. Eur Heart J 2014; 35:1917–1924.
7. Gomaraschi M, Adorni MP, Banach M, *et al.* Effects of established hypolipidemic drugs on HDL concentration, subclass distribution, and function. Handb Exp Pharmacol 2015; 224:593–615.
8. Hutchins PM, Heinecke JW. Cholesterol efflux capacity, macrophage reverse cholesterol transport and cardioprotective HDL. Curr Opin Lipidol 2015; 26:388–393.
9. ■ Du XM, Kim MJ, Hou L, *et al.* HDL particle size is a critical determinant of ABCA1-mediated macrophage cellular cholesterol export. Circ Res 2015; 116:1133–1142.

By using macrophages with defined expression of ABCA1 and ABCG1 as well as native and reconstituted HDL with defined size, the authors demonstrate that lipid-free apoA-I and small dense HDL particles are most efficient inducers of ABCA1-mediated cholesterol efflux. These particles are proposed to be the ideal targets for HDL-directed therapies.

10. ■ Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. Nat Rev Immunol 2015; 15:104–116.

An excellent review of interactions between cholesterol metabolism and innate immunity. The authors propose that cellular cholesterol accumulation enhances the inflammatory response of neutrophils and macrophages. This reaction could help in the defence of acute infections but triggers chronic inflammation and thereby promotes diseases like diabetes and atherosclerosis.

11. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014; 510:92–101.
12. Chung S, Parks JS. Dietary cholesterol effects on adipose tissue inflammation. Curr Opin Lipidol 2016; 27:19–25.
13. Mineo C, Shaul PW. Regulation of signal transduction by HDL. J Lipid Res 2013; 54:2315–2324.
14. Nofer JR. Signal transduction by HDL: agonists, receptors, and signalling cascades. Handb Exp Pharmacol 2015; 224:229–256.
15. ■■ Blaho VA, Galvani S, Engelbrecht E, *et al.* HDL-bound sphingosine-1-phosphate restrains lymphopoiesis and neuroinflammation. Nature 2015; 523:342–346.

ApoM knock-out mice showed increased proliferation of common hematopoietic progenitor cells as well as lymphoid progenitor cells in the bone marrow. This hyperproliferation could be inhibited by pharmacological activation or genetic overexpression of the S1P1 receptor. In-vitro apoM-bound S1P but not albumin-bound S1P suppressed lymphopoiesis. Experimental autoimmune encephalitis was more exacerbated in apoM knock-out mice than in wild-type mice. The authors suggest that HDL and apoM are therapeutic targets for the control of autoimmune diseases such as multiple sclerosis.

16. Lüscher TF, Landmesser U, von Eckardstein A, Fogelman AM. High-density lipoprotein: vascular protective effects, dysfunction, and potential as therapeutic target. Circ Res 2014; 114:171–182.
17. Siebel AL, Heywood SE, Kingwell BA. HDL and glucose metabolism: current evidence and therapeutic potential. Front Pharmacol 2015; 6:258.
18. Rye KA, Barter PJ, Cochran BJ. Apolipoprotein A-I interactions with insulin secretion and production. Curr Opin Lipidol 2016; 27:8–13.
19. Tan JT, Ng MK, Bursill CA. The role of high-density lipoproteins in the regulation of angiogenesis. Cardiovasc Res 2015; 106:184–193.
20. Van Linthout S, Frias M, Singh N, De Geest B. Therapeutic potential of HDL in cardioprotection and tissue repair. Handb Exp Pharmacol 2015; 224:527–565.
21. Proia RL, Hla T. Emerging biology of sphingosine-1-phosphate: its role in pathogenesis and therapy. J Clin Invest 2015; 125:1379–1387.
22. ■ Galvani S, Sanson M, Blaho VA, *et al.* HDL-bound sphingosine 1-phosphate acts as a biased agonist for the endothelial cell receptor S1P1 to limit vascular inflammation. Sci Signal 2015; 8:ra79.

Endothelium-specific knock out and overexpression of the S1P1 receptor increased and decreased, respectively, the expression of ICAM-1. In cultivated endothelial cells, apoM-bound S1P but not albumin-bound S1P suppressed TNFa-induced NF_kB activation and ICAM-1 expression. Atheorsclerosis of apoE−/− mice was enhanced by endothelium-specific knock out of S1P1. The authors suggest that S1P transported by HDL and bound by apoM limits vascular inflammation and explains the vasoprotectice functions of HDL at least partially.

23. Brulhart-Meynet MC, Braunersreuther V, Brinck J, *et al.* Improving reconstituted HDL composition for efficient postischemic reduction of ischemia reperfusion injury. PLoS One 2015; 10:e0119664.
24. Sutter I, Park R, Othman A, *et al.* Apolipoprotein M modulates erythrocyte efflux and tubular reabsorption of sphingosine-1-phosphate. J Lipid Res 2014; 55:1730–1737.
25. ■ Sattler K, Gräler M, Keul P, *et al.* Defects of high-density lipoproteins in coronary artery disease caused by low sphingosine-1-phosphate content: correction by sphingosine-1-phosphate-loading. J Am Coll Cardiol 2015; 66:1470–1485.

HDL of CHD patients and apoM knock-out mice are found to have reduced content of S1P and reduced capability to stimulate eNOS activation. The endothelial dysfunction of HDL from CHD patients as well as apoM knock-out mice could be restored by preloading with S1P both *in vitro* and *in vivo*.

26. Karlsson H, Kontush A, James RW. Functionality of HDL: antioxidation and detoxifying effects. Handb Exp Pharmacol 2015; 224:207–228.
27. Hussain MM. Intestinal lipid absorption and lipoprotein formation. Curr Opin Lipidol 2014; 25:200–206.

28. Rasmiena AA, Barlow CK, Ng TW, *et al.* High density lipoprotein efficiently accepts surface but not internal oxidised lipids from oxidised low density lipoprotein. Biochim Biophys Acta 2015; 1861: 69–77.
29. Soran H, Schofield JD, Liu Y, Durrington PN. How HDL protects LDL against atherogenic modification: paraoxonase 1 and other dramatis personae. Curr Opin Lipidol 2015; 26:247–256.
30. Chung DW, Chen J, Ling M, *et al.* High density lipoprotein modulates thrombosis by preventing von Willebrand factor self-association and subsequent platelet adhesion. Blood 2016; 127:637–645.
31. Niyonzima N, Samstad EO, Aune MH, *et al.* Reconstituted high-density lipoprotein attenuates cholesterol crystal-induced inflammatory responses by reducing complement activation. J Immunol 2015; 195:257–264.
32. Kontush A, Lindahl M, Lhomme M, *et al.* Structure of HDL: particle subclasses and molecular components. Handb Exp Pharmacol 2015; 224:3–51.
33. Moren X, Lhomme M, Bulla A, *et al.* Proteomic and lipidomic analyses of paraoxonase defined high density lipoprotein particles: association of paraoxonase with the anticoagulant, protein S. Proteomics Clin Appl 2016; 10:230–238.
34. Vickers KC, Palmisano BT, Shoucri BM, *et al.* MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 2011; 13:423–433.
35. Tabet F, Vickers KC, Cuesta Torres LF, *et al.* HDL-transferred microRNA-223 regulates ICAM-1 expression in endothelial cells. Nat Commun 2014; 5:3292.
36. Wagner J, Riwanto M, Besler C, *et al.* Characterization of levels and cellular transfer of circulating lipoprotein-bound microRNAs. Arterioscler Thromb Vasc Biol 2013; 33:1392–1400.
37. Schober A, Nazari-Jahantigh M, Weber C. MicroRNA-mediated mechanisms of the cellular stress response in atherosclerosis. Nat Rev Cardiol 2015; 12:361–374.
38. Röhrl C, Stangl H. HDL endocytosis and resecretion. Biochim Biophys Acta 2013; 1831:1626–1633.
39. Martinez LO, Najib S, Perret B, *et al.* Ecto-F1-ATPase/P2Y pathways in metabolic and vascular functions of high density lipoproteins. Atherosclerosis 2015; 238:89–100.
40. ■ Lichtenstein L, Serhan N, Espinosa-Delgado S, *et al.* Increased atherosclerosis in P2Y13/apolipoprotein E double-knock-out mice: contribution of P2Y13 to RCT. Cardiovasc Res 2015; 106:314–323.

The authors previously showed that activation of the purinergic receptor P2Y13 stimulates HDL-holoparticle uptake in hepatocytes. They now report the phenotype of apoE$-/-\times$P2Y13$-/-$ double knock-out mice. These mice show increased atherosclerosis, lower HDL-C levels (possibly because of upregulation of SR-B1), and decreased macrophage RCT. Bone marrow transplantation experiments revealed that the phenotype is caused by the lack of P2Y13 from nonmyeloid cells. In conclusion, the data support an important role of P2Y13 in RCT, possibly by regulating HDL-holoparticle uptake into the liver.

41. Genoux A, Ruidavets JB, Ferrières J, *et al.* Serum IF1 concentration is independently associated to HDL levels and to coronary heart disease: the GENES study. J Lipid Res 2013; 54:2550–2558.
42. Aseem O, Smith BT, Cooley MA, *et al.* Cubilin maintains blood levels of HDL and albumin. J Am Soc Nephrol 2014; 25:1028–1036.
43. Perisa D, Rohrer L, Kaech A, von Eckardstein A. Itinerary of high density lipoproteins in endothelial cells. Biochim Biophys Acta 2016; 1861:98–107.
44. Meilhac O. High-density lipoproteins in stroke. Handb Exp Pharmacol 2015; 224:509–526.
45. ■ Gerster R, Eloranta JJ, Hausmann M, *et al.* Anti-inflammatory function of high-density lipoproteins via autophagy of IκB kinase. Cell Mol Gastroenterol Hepatol 2015; 1:171–187.

Dextran sodium sulfate induces colitis in wild-type mice. This phenotype was found enhanced in apoA-I knock-out mice and decreased in apoA-I transgenic mice. *In vitro*, HDL and apoA-I inhibited TNFα-induced mRNA expression of TNFα, IL-8, and ICAM, by preventing NFκB activation. As a novel antiinflammatory mechanism, HDL was found to induce the recruitment of phosphorylated IκB kinase to the autophagosome compartment.

46. Cheng D, Weckerle A, Yu Y, *et al.* Biogenesis and cytotoxicity of APOL1 renal risk variant proteins in hepatocytes and hepatoma cells. J Lipid Res 2015; 56:1583–1593.
47. Rosenson RS, Brewer HB Jr, Ansell BJ, *et al.* Dysfunctional HDL and atherosclerotic cardiovascular disease. Nat Rev Cardiol 2016; 13:48–60.
48. Sutter I, Velagapudi S, Othman A, *et al.* Plasmalogens of high-density lipoproteins (HDL) are associated with coronary artery disease and antiapoptotic activity of HDL. Atherosclerosis 2015; 241:539–546.
49. Besler C, Heinrich K, Rohrer L, *et al.* Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Invest 2011; 121:2693–2708.
50. Speer T, Rohrer L, Blyszczuk P, *et al.* Abnormal high-density lipoprotein induces endothelial dysfunction via activation of Toll-like receptor-2. Immunity 2013; 38:754–768.
51. ■ Zewinger S, Drechsler C, Kleber ME, *et al.* Serum amyloid A: high-density lipoproteins interaction and cardiovascular risk. Eur Heart J 2015; 36:3007–3016.

In three prospective cohort studies, plasma levels of SAA were found to modify the cardiovascular risk associated with low HDL-C. In the presence of elevated SAA levels, HDL-C was positively rather than inversely associated with risk of incident fatal or nonfatal CHD. Accompanying in-vitro experiments revealed that enrichment of SAA turns an HDL particle with endothelium protective functions into a noxious particle that inhibits rather than stimulates nitric oxide production and that stimulates rather than inhibits adhesion molecule expression.

52. Camont L, Lhomme M, Rached F, *et al.* Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: relevance to cellular cholesterol efflux, antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic functionalities. Arterioscler Thromb Vasc Biol 2013; 33:2715–2723.
53. Ronsein GE, Hutchins PM, Isquith D, *et al.* Niacin therapy increases high-density lipoprotein particles and total cholesterol efflux capacity but not ABCA1-specific cholesterol efflux in statin-treated subjects. Arterioscler Thromb Vasc Biol 2016; 36:404–411.
54. Borja MS, Ng KF, Irwin A, *et al.* HDL-apolipoprotein A-I exchange is independently associated with cholesterol efflux capacity. J Lipid Res 2015; 56:2002–2009.
55. Agarwala AP, Rodrigues A, Risman M, *et al.* High-density lipoprotein (HDL) phospholipid content and cholesterol efflux capacity are reduced in patients with very high HDL cholesterol and coronary disease. Arterioscler Thromb Vasc Biol 2015; 35:1515–1519.
56. Zheng L, Settle M, Brubaker G, *et al.* Localization of nitration and chlorination sites on apolipoprotein A-I catalyzed by myeloperoxidase in human atheroma and associated oxidative impairment in ABCA1-dependent cholesterol efflux from macrophages. J Biol Chem 2005; 280:38–47.
57. Huang Y, DiDonato JA, Levison BS, *et al.* An abundant dysfunctional apolipoprotein A1 in human atheroma. Nat Med 2014; 20:193–203.
58. Hewing B, Parathath S, Barrett T, *et al.* Effects of native and myeloperoxidase-modified apolipoprotein a-I on reverse cholesterol transport and atherosclerosis in mice. Arterioscler Thromb Vasc Biol 2014; 34:779–789.
59. Vaisar T, Tang C, Babenko I, *et al.* Inflammatory remodeling of the HDL proteome impairs cholesterol efflux capacity. J Lipid Res 2015; 56:1519–1530.
60. ■ Han CY, Tang C, Guevara ME, *et al.* Serum amyloid A impairs the antiinflammatory properties of HDL. J Clin Invest 2016; 126:796.

The authors induced SAA production in mice by injection of silver nitrate. The sterile inflammation compromised the ability of HDL from wild-type mice but not from SAA1.1$-/-\times$SAA2.1$-/-$ double knock-out mice to stimulate cholesterol-efflux and to inhibit palmitate-induced expression of inflammatory genes in adipocytes. The phenotypes were imitated by HDL from mice or human patients with inflammatory diseases as well as by loading of normal HDL with SAA. Enzymatic digestion of proteoglycans in the extracellular matrix of adipocytes restored the function of dysfunctional HDL.

61. Silbernagel G, Schöttker B, Appelbaum S, *et al.* High-density lipoprotein cholesterol, coronary artery disease, and cardiovascular mortality. Eur Heart J 2013; 34:3563–3571.
62. Schwartz GG, Olsson AG, Abt M, Wright RS, *et al.,* dal-OUTCOMES Investigators. Effects of dalcetrapib in patients with a recent acute coronary syndrome. N Engl J Med 2012; 367:2089–2099.
63. ■ Colantonio LD, Bittner V, Reynolds K, *et al.* Association of serum lipids and coronary heart disease in contemporary observational studies. Circulation 2016; 133:256–264.

A valuable study of secular trends in the prognostic performance of lipid risk factors. The risk associations of LDL-C, non-HDL-C, triglycerides, and HDL-C were significantly weaker in populations examined in this millennium as compared with the ARIC population examined in the 1980s. The authors explain the risk attenuation as the consequence of increasing statin treatment.

64. Miller WG, Myers GL, Sakurabayashi I, *et al.* Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. Clin Chem 2010; 56:977–986.
65. Langlois MR, Descamps OS, van der Laarse A, *et al.,* EAS-EFLM Collaborative Project. Clinical impact of direct HDLc and LDLc method bias in hypertriglyceridemia. A simulation study of the EAS-EFLM Collaborative Project Group. Atherosclerosis 2014; 233:83–90.
66. Sacks FM, Carey VJ. J.C. Fruchart Combination lipid therapy in type 2 diabetes. N Engl J Med 2010; 363:692–694.
67. ■ Tardif JC, Rheaume E, Lemieux Perreault LP, *et al.* Pharmacogenomic determinants of the cardiovascular effects of dalcetrapib. Circ Cardiovasc Genet 2015; 8:372–382.

A genome-wide association study identified single nucleotide polymorphisms in the ADCY9 gene as discriminators of treatment response to dalcetrapib. Dalcetrapib treatment reduced event rates in carriers of the AA allele by 39% but increased event rates in carrier of the GG allele in rs1967309. ADCY9 encodes adenylyl cyclase which mediates β2-adrenoreceptor signaling. The genetic association of treatment response to dalcetrapib points to an interaction of functional or dysfunctional HDL with this signaling platform.

68. Kathiresan S. Will cholesteryl ester transfer protein inhibition succeed primarily by lowering low-density lipoprotein cholesterol? Insights from human genetics and clinical trials. J Am Coll Cardiol 2012; 60:2049–2052.

69. Niu W, Qi Y. Circulating cholesteryl ester transfer protein and coronary heart disease: mendelian randomization meta-analysis. Circ Cardiovasc Genet 2015; 8:114–121.

70. https://investor.lilly.com/releasedetail.cfm?ReleaseID=936130. (Accessed 28 December 2015)

71. Reina SA, Llabre MM, Allison MA, *et al.* HDL cholesterol and stroke risk: the multi-ethnic study of atherosclerosis. Atherosclerosis 2015; 243:314–319.

72. Burillo E, Lindholt JS, Molina-Sánchez P, *et al.* ApoA-I/HDL-C levels are inversely associated with abdominal aortic aneurysm progression. Thromb Haemost 2015; 113:1335–1346.

73. Haase CL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. HDL cholesterol and risk of type 2 diabetes: a Mendelian randomization study. Diabetes 2015; 64:3328–3333.

74. GENESIS Consortium. Fall T, Xie W, Poon W, *et al.* Using genetic variants to assess the relationship between circulating lipids and type 2 diabetes. Diabetes 2015; 64:2676–2684.

75. Ni H, Liu H, Gao R. Serum lipids and breast cancer risk: a meta-analysis of prospective cohort studies. PLoS One 2015; 10:e0142669.

76. van Duijnhoven FJ, Bueno-De-Mesquita HB, Calligaro M, *et al.* Blood lipid and lipoprotein concentrations and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition. Gut 2011; 60:1094–1102.

77. Morin EE, Guo L, Schwendeman A, Li XA. HDL in sepsis: risk factor and therapeutic approach. Front Pharmacol 2015; 6:244.

78. Yip JL, Khawaja AP, Chan MP, *et al.* Cross sectional and longitudinal associations between cardiovascular risk factors and age related macular degeneration in the EPIC-Norfolk eye study. PLoS One 2015; 10:e0132565.

79. Cheng CY, Yamashiro K, Chen LJ, *et al.* New loci and coding variants confer risk for age-related macular degeneration in East Asians. Nat Commun 2015; 6:6063.

80. Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. Circulation 2007; 115:949–952.

81. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation 2006; 113:2335–2362.

82. Rosenson RS, Brewer HB Jr, Chapman MJ, *et al.* HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. Clin Chem 2011; 57:392–410.

83. Kontush A. HDL particle number and size as predictors of cardiovascular disease. Front Pharmacol 2015; 6:218.

84. Kuller LH, Grandits G, Cohen JD, *et al.*, Multiple Risk Factor Intervention Trial Research Group. Lipoprotein particles, insulin, adiponectin, C-reactive protein and risk of coronary heart disease among men with metabolic syndrome. Atherosclerosis 2007; 195:122–128.

85. Mackey RH, Greenland P, Goff DC Jr, *et al.* High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multiethnic study of atherosclerosis). J Am Coll Cardiol 2012; 60:508–516.

86. Chandra A, Neeland IJ, Das SR, *et al.* Relation of black race between high density lipoprotein cholesterol content, high density lipoprotein particles and coronary events (from the Dallas Heart Study). Am J Cardiol 2015; 115:890–894.

87. Mora S, Glynn RJ, Ridker PM. High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. Circulation 2013; 128:1189–1197.

88. Mora S, Otvos JD, Rifai N, *et al.* Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. Circulation 2009; 119:931–939.

89. Qi Y, Fan J, Liu J, *et al.* Cholesterol-overloaded HDL particles are independently associated with progression of carotid atherosclerosis in a cardiovascular disease-free population: a community-based cohort study. J Am Coll Cardiol 2015; 65:355–363.

90. El Harchaoui K, Arsenault BJ, Franssen R, *et al.* High-density lipoprotein particle size and concentration and coronary risk. Ann Intern Med 2009; 150:84–93.

91. Steffen BT, Guan W, Remaley AT, *et al.* Use of lipoprotein particle measures for assessing coronary heart disease risk post-American Heart Association/American College of Cardiology guidelines: the multi-ethnic study of atherosclerosis. Arterioscler Thromb Vasc Biol 2015; 35:448–454.

92. Mora S, Caulfield MP, Wohlgemuth J, *et al.* Atherogenic lipoprotein subfractions determined by ion mobility and first cardiovascular events after random allocation to high-intensity statin or placebo: the justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin (JUPITER) trial. Circulation 2015; 132:2220–2229.

93. Mallol R, Amigó N, Rodríguez MA, *et al.* Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. J Lipid Res 2015; 56:737–746.

94. Kaess BM, Tomaszewski M, Braund PS, *et al.* Large-scale candidate gene analysis of HDL particle features. PLoS One 2011; 6:e14529.

95. Würtz P, Raiko JR, Magnussen CG, *et al.* High-throughput quantification of circulating metabolites improves prediction of subclinical atherosclerosis. Eur Heart J 2012; 33:2307–2316.

96. Khera AV, Cuchel M, de la Llera-Moya M, *et al.* Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med 2011; 364:127–135.

97. ■ Rohatgi A, Khera A, Berry JD, *et al.* HDL-C efflux capacity and incident cardiovascular events. N Engl J Med 2014; 371:2383–2393.

The study for the first time shows an independent risk association of CEC with incident CHD.

98. O'Neill F, McLoughlin E, Riwanto M, *et al.* Reproducibility and biological variability of HDL's vascular functional assays. Atherosclerosis 2015; 241:588–594.

99. ■ Ritsch A, Scharnagl H, März W. HDL cholesterol efflux capacity and cardiovascular events. N Engl J Med 2015; 372:1870–1871.

A letter to Ref. [96] confirming the independent risk association of CEC with incident CHD in a clinical study cohort.

100. ■ Saleheen D, Scott R, Javad S, *et al.* Association of HDL cholesterol efflux capacity with incident coronary heart disease events: a prospective case-control study. Lancet Diabetes Endocrinol 2015; 3:507–513.

A nested-case control study confirming the independent risk association of CEC with incident CHD.

101. Li XM, Tang WH, Mosior MK, *et al.* Paradoxical association of enhanced cholesterol efflux with increased incident cardiovascular risks. Arterioscler Thromb Vasc Biol 2013; 33:1696–1705.

102. ■ Annema W, Dikkers A, de Boer JF, *et al.* HDL-C efflux predicts graft failure in renal transplant recipients. J Am Soc Nephrol 2016; 27:595–603.

CKD patients have a high risk of cardiovascular events also after kidney transplantation. In this study CEC was not associated with the incidence of fatal or noncardiovascular events or total mortality but with graft failure independently of potential confounders including HDL-C or apoA-I levels.

103. Nicholls SJ, Ruotolo G, Brewer HB, *et al.* Cholesterol efflux capacity and pre-beta-1 HDL concentrations are increased in dyslipidemic patients treated with evacetrapib. J Am Coll Cardiol 2015; 66:2201–2210.

104. Ballantyne CM, Miller M, Niesor EJ, *et al.* Effect of dalcetrapib plus pravastatin on lipoprotein metabolism and high-density lipoprotein composition and function in dyslipidemic patients: results of a phase IIb dose-ranging study. Am Heart J 2012; 163:515–552.

105. Munroe WH, Phillips ML, Schumaker VN. Excessive centrifugal fields damage high density lipoprotein. J Lipid Res 2015; 56:1172–1181.

106. Wyler von Ballmoos MC, Haring B, Sacks FM. The risk of cardiovascular events with increased apolipoprotein CIII: A systematic review and meta-analysis. J Clin Lipidol 2015; 9:498–510.

107. Kopecky C, Genser B, Drechsler C, *et al.* Quantification of HDL proteins, cardiac events, and mortality in patients with type 2 diabetes on hemodialysis. Clin J Am Soc Nephrol 2015; 10:224–231.

108. Silbernagel G, Genser B, Drechsler C, *et al.* HDL cholesterol, apolipoproteins, and cardiovascular risk in hemodialysis patients. J Am Soc Nephrol 2015; 26:484–492.

109. Duong M, Di Bartolo BA, Nicholls SJ. Acute HDL therapies. Curr Opin Lipidol 2015; 26:521–525.